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Hepatic-Portal Vein Infusions of Glucagon-Like Peptide-1 Reduce Meal Size and Increase c-Fos Expression in the Nucleus Tractus Solitarii, Area Postrema and Central Nucleus of the Amygdala in Rats

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Zusammenfassung

Die Infusion von Glucagon-Like Peptide-1 in die Pfortader reduziert die Mahlzeitgrösse und erhöht die Expression von c-Fos im Nucleus tractus solitarii, in der Area postrema und im Zentralkern der Amygdala bei Ratten

Kürzlich berichteten wir, dass kurze, ferngesteuerte Infusionen von Glucagon-Like Peptide-1 (GLP-1) in die Pfortader von Ratten während einer Mahlzeit die spontane Mahlzeitengrösse reduzieren. Um die verhaltensneurologischen Wechselwirkungen dieses Effekts zu untersuchen, implantierten wir männlichen Sprague-Dawley Ratten Pfortaderkatheter und erfassten den Einfluss von ferngesteuerten Infusionen von GLP-1 (1 nmol/kg, 5 min) oder Kontrolllösung auf den Verzehr während der ersten Mahlzeit in der Dunkelphase nach 3h Futterentzug. Ferner erfassten wir den Einfluss analoger GLP-1 Infusionen am Beginn der Dunkelphase auf die c-Fos Expression in mehreren Gehirnarealen, welche in die Steuerung des Verzehrs involviert sind. GLP-1 reduzierte ($P < 0.05$) die Grösse der ersten Mahlzeit in der Dunkelphase und steigerte deren Sättigungsquotienten (Dauer des nachfolgenden Mahlzeitenintervals [min]/Mahlzeitgrösse [g]). Ebenso vermehrte ($P < 0.05$) GLP-1 die Anzahl der c-Fos exprimierenden Zellen im Nucleus tractus solitarii, in der Area postrema und im Zentralkern der Amygdala, nicht aber im Nucleaus arcuatus oder im Nucleus paraventricularis des Hypothalamus. Diese Daten lassen darauf schliessen, dass der Nucleus tractus solitarii, die Area postrema und der Zentralkern der Amygdala für den verzehrshemmenden Effekt von GLP-1 nach Infusion in die Pfortader eine Rolle spielen. Es bleibt festzustellen, ob die Aktivierung dieser Hirnareale Sättigung, Aversion oder beides reflektiert.

Schlüsselwörter: Verzehr, Sättigung, intestinales Peptid, Hinterhirn, Aversion

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ORIGINAL ARTICLE

Hepatic-Portal Vein Infusions of Glucagon-Like Peptide-1 Reduce Meal Size and Increase c-Fos Expression in the Nucleus Tractus Solitarii, Area Postrema and Central Nucleus of the Amygdala in Rats

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We recently reported that brief, remotely controlled intrameal hepatic-portal vein infusions of glucagon-like peptide-1 (GLP-1) reduced spontaneous meal size in rats. To investigate the neurobehavioural correlates of this effect, we equipped male Sprague-Dawley rats with hepatic-portal vein catheters and assessed (i) the effect on eating of remotely triggered infusions of GLP-1 (1 nmol/kg, 5 min) or vehicle during the first nocturnal meal after 3 h of food deprivation and (ii) the effect of identical infusions performed at dark onset on c-Fos expression in several brain areas involved in the control of eating. GLP-1 reduced ($P < 0.05$) the size of the first nocturnal meal and increased its satiety ratio. Also, GLP-1 increased ($P < 0.05$) the number of c-Fos-expressing cells in the nucleus tractus solitarii, the area postrema and the central nucleus of the amygdala, but not in the arcuate or paraventricular hypothalamic nuclei. These data suggest that the nucleus tractus solitarii, the area postrema and the central nucleus of the amygdala play a role in the eating-inhibitory actions of GLP-1 infused into the hepatic-portal vein; it remains to be established whether activation of these brain nuclei reflect satiation, aversion, or both.

Key words: eating, satiation, gut peptide, hindbrain, aversion.

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Glucagon-like peptide-1-(7-36)-amide (GLP-1) is synthesised and released by L-cells in the distal ileum and colon and by a small population of neurones in the nucleus tractus solitarii (NTS) (1, 2). GLP-1 receptors (GLP-1R) are widely expressed both in the brain and in the periphery, where they are found in the gastrointestinal tract, pancreatic islets, portal vein, liver and vagus nerve (1, 2). GLP-1 is considered to have a range of physiological actions, including stimulation of insulin secretion (i.e. an incretin effect) (1, 2), inhibition of glucagon secretion (1, 3), inhibition of gastrointestinal secretory and motor functions (i.e. ileal brake effect) (4), control of autonomic and behavioural stress and aversion responses (5, 6) and inhibition of eating (1, 6–8).

The effects of GLP-1 on eating include specific reductions in meal size ('satiation') (5, 8–11) and nonspecific anorexia (e.g. the anorexia produced by the visceral malaise that both directly inhibits eating and leads to the formation of conditioned taste aversions) (5, 10, 12–15). The conditions under which (endogenous) GLP-1 produces satiation or malaise-related anorexia, and the relative contributions of peripheral and central GLP-1 and GLP-1R to these actions, remain unclear. Perhaps the strongest evidence that peripheral GLP-1 can have a specific satiating effect comes from the observations that i.p. administration of the GLP-1 antagonist exendin (9–39) acutely increased eating in rats under some conditions (11) and that i.v. infusion of physiological doses of GLP-1 (i.e. doses that mimic the increase in endogenous GLP-1 following meals) were sufficient to decrease food intake in humans without side effects (7, 16).

Recent findings from our laboratory indicate that peripheral GLP-1 acts at two different sites to inhibit eating in rats (9). In tests of brief, remotely controlled, infusions of 10 nmol/kg (33 μg/kg) GLP-1 during spontaneous meals, the eating-inhibitory effect of i.p. infused GLP-1 depended on intact abdominal vagal afferents, whereas the effect of hepatic-portal vein (HPV) infusions of GLP-1 did not. These data demonstrate that vagal afferent signalling is not necessary for the eating-inhibitory effect of HPV GLP-1 and are consistent with the idea that i.p. GLP-1, perhaps similar to endogenous GLP-1, inhibits eating by activating GLP-1R on vagal afferents terminating in the lamina propria of the intestinal mucosa. We also

observed that HPV GLP-1 infusions failed to inhibit eating more potently than vena caval infusions of the same GLP-1 dose, indicating that the GLP-1R mediating the eating-inhibitory effect of HPV GLP-1 are not located in the HPV area or in the liver. A recent study finding that GLP-1 inhibited eating more after intrajugular than after HPV infusion also supports this interpretation (17). Taken together, these data suggest that circulating GLP-1 may act on GLP-1R in the brain to inhibit eating.

Brain areas involved in processing of satiation signals are commonly investigated using c-Fos immunohistochemistry (IHC). As reviewed below, there are few studies associating the effects of GLP-1 on eating and c-Fos expression. In particular, the effects of i.v. administration of GLP-1 on c-Fos expression in the brain have not been characterised. Therefore, in the present study we aimed to determine the effects of HPV infusion of 1 nmol/kg GLP-1, a moderate dose that reliably reduced spontaneous meal size under our conditions (9), on neural activation, as measured by c-Fos IHC, in several brain areas involved in the control of eating.

Materials and methods

Subjects and housing

Male Sprague-Dawley [Crl:CD® (SD)] rats (Charles River, Sulzfeld, Germany), weighing 180–200 g body weight upon arrival, were individually housed in acrylic infusion cages [21 × 37 × 41 cm, length (l) × width (w) × height (h)] with stainless steel grid floors under a 12 : 12 h dark/light cycle (lights off 12.00 h) in a climate-controlled room (22 ± 2 °C and 60% relative humidity). Ground chow (3433; Provimi Kliba SA, Kaiseraugst, Switzerland) was available in food cups that were accessible in a niche [5 × 7 × 30 cm (l × w × h)], 6 cm above the cage floor, on one side of the cage. Tap water was available *ad lib.* A 60-W red incandescent light bulb provided dim illumination during the dark phase, and a radio tuned to a music station was played continuously to mask extraneous noise. All protocols were approved by the Veterinary Office of the Canton of Zurich.

Catheter implantation and patency checks

Two weeks after arrival, chronic HPV infusion catheters were implanted in all rats, as described previously (9). Catheters were flushed with 0.2 ml of 0.9% sterile saline and filled with 80 μl of heparinised saline (100 IU heparin/ml saline; Heparin; Braun, Melsungen, Germany) daily the first week after surgery and every 3 days thereafter. Catheter patency was verified by infusing 0.8 ml/kg body weight of a mixture of 26 mg/kg ketamine (Ketasol-100; Dr E. Gräub AG, Bern, Switzerland) and 0.9 mg/kg xylazine (Rompun; Bayer, Leverkusen, Germany) at the end of behavioural tests. The criterion for catheter patency was a complete loss of muscle tone within 1 min. In addition, in Experiment 2, 0.5 ml of Giemsa stain (Sigma-Aldrich, Buchs, Switzerland) was infused into the HPV after laparotomy, and the perfusion of the HPV with blue stain without leakage was verified. In Experiment 2, the criteria for catheter patency were that both the anesthesia test and the Giemsa stain tests be successful. Data from rats without patent HPV catheters were not included in the analyses.

Experiment 1: eating

Tests began 2 weeks after surgery. Food cups were mounted on electronic balances (XS4001S; Mettler-Toledo, Greifensee, Switzerland) linked to a computer that sampled the weight of each balance every 30 s. Meal patterns were analysed with custom-made software (LabX-Meal-analyzer 1.4; Mettler-Toledo). In addition, rats were monitored with infrared video cameras (Conrad Electronic GmbH, Hirschau, Germany). A meal was defined as a decrease of > 0.3 g in food cup weight and visual verification of eating. Experimenters in an adjacent control room monitored the balance weights and rats' behaviour and triggered the infusions by remote control. Twelve rats (body weight = 346 ± 4 g) were adapted to the following procedure for several days: (i) 08.15 h, weigh rats; (ii) 09.00 h, remove food; (iii) 11.00 h, attach HPV catheters to the infusion pumps as described previously (9); (iv) 12.00 h, offer fresh ground chow; (v) 2 min after the onset of the first meal, start HPV infusion (0.2 ml/min for 5 min) of 1 nmol/kg GLP-1(7-36)-amide (Bachem, Bubendorf, Switzerland) dissolved in phosphate-buffered saline (PBS) with 1% bovine serum albumin (Sigma-Aldrich) or vehicle; and (vi) 16.00 h, disconnect rats from infusion pumps and flush catheters. GLP-1 and vehicle were tested on consecutive days using a within-subjects cross-over design.

Experiment 2: c-Fos immunohistochemistry

Sixteen rats underwent similar surgical procedures as for Experiment 1. Two weeks after surgery, rats were adapted to the infusion protocol and to a 19-h feeding – 5-h (09.00–14.00 h) food deprivation schedule. The experiment was performed after a few weeks of adaptation, when rats weighed 442 ± 11 g. GLP-1 (1 nmol/kg body weight, n = 9) or vehicle (n = 7) was infused (0.2 ml/min, 5 min) at the onset of dark (12.00 h, food was not returned) and, 90 min later, rats were deeply anesthetised with an i.p. injection of 100 mg/kg body weight sodium pentobarbital (Cantonal Pharmacy, Zurich, Switzerland) and transcardially perfused with PBS (0.1 M, pH 7.2) followed by 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA). Infusions and perfusions were performed at 5-min intervals within 25 min of the times given above. Brains were excised and post-fixed overnight at 4 °C, then switched to 30% sucrose in 0.1 M PBS, and kept at 4 °C until processing.

Brains were sectioned using a freezing sliding microtome (Microm International, Walldorf, Germany) and 40-μm sections were collected and stored in 0.1 M PBS with 0.1% sodium azide (Fluka, Buchs, Switzerland) at 4 °C. Every sixth section of the forebrain (i.e. each 240 μm) and every twelfth section of the hindbrain (i.e. each 480 μm) were processed for c-Fos using a standard single-label indirect IHC technique (18). Briefly, after endogenous peroxidase quenching (0.5% H_2O_2, 0.1 M PBS, 30 min, at room temperature; RT), and blocking nonspecific interactions and permeabilisation (5% normal goat serum, 0.1 M PBS, 0.3% Triton-X, 1 h, RT), brain sections were incubated with primary antibody (1 : 2500 rabbit anti-c-Fos; 72 h, 4°C, sc-52; Santa Cruz Biotech., Santa Cruz, CA, USA). Afterwards, sections were incubated with a secondary biotinylated antibody (1 : 200 goat anti-rabbit IgG; 2 h, RT; Vector Laboratories, Burlingame, CA, USA). The antibody complex was visualised by allowing the streptavidin-diaminobenzidine (DAB)-peroxidase (ABC kit; Vector Laboratories) to react with the DAB-tetrahydrochloride substrate (5 min, RT; Sigma-Aldrich). All incubation steps were performed on an orbital shaker at approximately 250 Hz, and several interim rinse steps were performed throughout the procedure. Sections were then mounted on gelatin-coated slides, dehydrated in an increasing series of alcohols, defatted in xylene, and coverslipped with Permount (Menzel, Braunschweig, Germany). Photomicrographs were digitally acquired under × 20 magnification and bright-field illumination using an Olympus AX-70 microscope (Olympus, Center Valley, PA, USA) connected to a digital colour camera interfaced to a computer. Photomicrographs were assigned to the nearest anterior-posterior bregma level according to a rat brain atlas (19), and c-Fos-positive cells were counted within the regions of interest (ROI). Transparencies made from the brain atlas were used to delineate ROI at the closest anterior-posterior level, and cell counting was performed using VOLOCITY 4 software

(Improvision, Coventry, UK; settings: 65–85 % of intensity, 30 μm^2 < object size > 200 μm^2), operated by an experimenter who was blind to the treatments. Brain structures counted were: NTS (six sections between 13.08 and 15.48 mm posterior to bregma), area postrema (AP; one section at approximately 14.04 mm posterior), central nucleus of the amygdala (CeA; eight sections between 1.56 and 3.24 mm posterior), arcuate nucleus (nine sections between 1.72 and 3.64 mm posterior), and hypothalamic paraventricular nucleus (PVN; eight sections between 0.72 and 2.04 mm posterior).

Statistical analysis

Meal sizes (first and second) during the test period, intermeal interval (IMI) (duration between the end of the first meal and the start of the second meal), and first meal satiety ratio (IMI/first meal size) were analysed with paired-sample t-tests using SPSS, version 16.0 (SPSS Inc., Chicago, IL, USA). c-Fos-positive cell counts in the anterior-posterior regions of the NTS, CeA, arcuate nucleus and PVN in which c-Fos-positive cells were detected were analysed by two-way ANOVA with treatment (GLP-1, vehicle) and bregma level as factors. Significant ANOVA interaction effects were followed-up with Bonferroni-Holm contrasts (20) between GLP-1- and vehicle-treated rats at each bregma level. Because only one section in the AP was counted, c-Fos-positive cells in this area were analysed using an independent-sample t-test. To increase statistical power, data were converted to standard scores using the median absolute deviate method (21). Extreme values, defined as standard scores with absolute values > 1.96 (i.e. $P < 0.05$), were excluded. The incidence of such values was: NTS, 5%; AP, 13%; CeA, 23%; arcuate nucleus, 17%; and PVN 10%. A nonparametric reanalysis of the complete data sets yielded the same effects as the post-hoc parametric analysis. Data are presented as the mean ± SEM, and the standard error of the difference (SED) is given as a measure of residual error variability. $P < 0.05$ was considered statistically significant.

Results

Experiment 1: eating

HPV infusions of 1 nmol/kg GLP-1 reduced the size of the first meal ($t_8 = 2.82$, $P = 0.02$, SED = 0.6 g) (Fig. 1A) and increased the first meal satiety ratio ($t_8 = 4.16$, $P = 0.003$, SED = 3.8 min/g) versus vehicle treatment (Fig. 1B). The IMI between the first and second meals ($t_7 = 0.34$, $P > 0.05$, SED = 17 min) and the size of the second meal ($t_7 = 1.39$, $P > 0.05$, SED = 0.6 g) were not significantly affected by GLP-1 (Fig. 1C, D). The analyses were performed on ten rats that passed the catheter patency test.

Experiment 2: c-Fos immunohistochemistry

HPV GLP-1 infusion increased the number of c-Fos-positive cells in the NTS between 13.08 and 14.52 mm posterior to bregma (interaction effect: $F_{3,36} = 4.06$, $P < 0.001$, SED = 10 cells/section), although post-hoc tests detected a significant difference between GLP-1- and vehicle-treated rats only at 14.04 mm posterior to bregma (Fig. 2A). HPV GLP-1 infusion significantly increased the number of c-Fos-positive cells in the AP at this level as well (Fig. 2B; $t_{11} = -2.63$, $P = 0.02$, SED = 28 cells/section). Representative examples of c-Fos staining in the NTS and AP are given in Fig. 2(C, D).

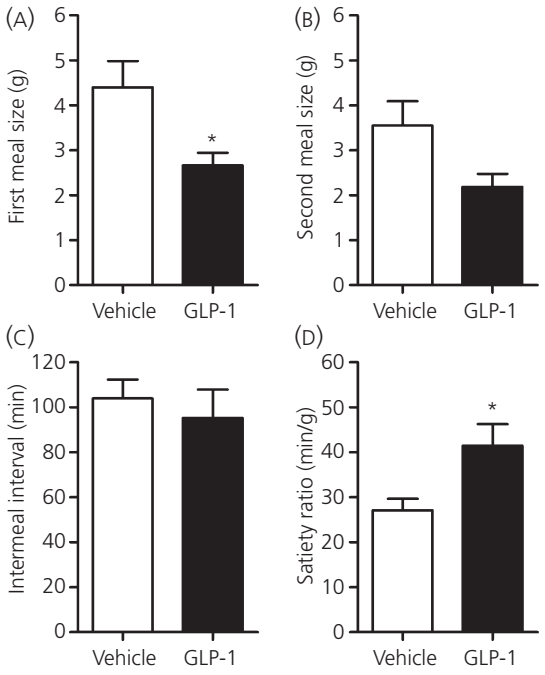

Fig. 1. Intrameal hepatic-portal vein infusions of 1 nmol/kg glucagon-like peptide-1 (GLP-1) reduced the size of the first meal compared to vehicle infusions (A), but not of the second meal (B). The intermeal interval between the first and the second meal was not affected by GLP-1 (C), but the first meal satiety ratio was increased (D). Values are the mean ± SEM of ten rats. *$P < 0.05$.

HPV GLP-1 infusion also increased the number of c-Fos-positive cells in the CeA between 2.28 and 3.24 mm posterior to bregma, which reached statistical significance at 2.52 and 3.00 mm posterior to bregma (Fig. 3A; treatment effect: $F_{1,13} = 8.78$, $P = 0.006$, bregma level effect: $F_{5,44} = 8.78$, $P < 0.001$, SED = 15 cells/section). A significant interaction effect was found for the number of c-Fos-positive cells in the arcuate nucleus ($F_{8,81} = 3.13$, $P < 0.001$, SED = 9 cells/section). Post-hoc tests detected significant difference only at 3.16 mm posterior to bregma, which was probably the result of an unusually high number of c-Fos-positive cells in vehicle-treated rats because no such trend was observed in the adjacent sections (Fig. 3B). Finally, HPV GLP-1 infusion did not affect the number of c-Fos-positive cells in the PVN (Fig. 3C). More c-Fos-positive cells were found at posterior levels of the PVN (bregma level effect: $F_{6,67} = 13.30$, $P < 0.001$, SED = 12 cells/section), although there was no reliable treatment or interaction effect. c-Fos expression analyses were performed on fifteen rats that passed the catheter-patency tests. Representative examples of c-Fos staining in the CeA, arcuate nucleus and PVN are given in Fig. 3(D–I).

Discussion

The main novel finding of the present study is that the satiating action of intrameal HPV infusion of 1 nmol/kg GLP-1 in rats is associated with increased expression of c-Fos in the NTS, AP and CeA, but not in the arcuate nucleus or PVN.

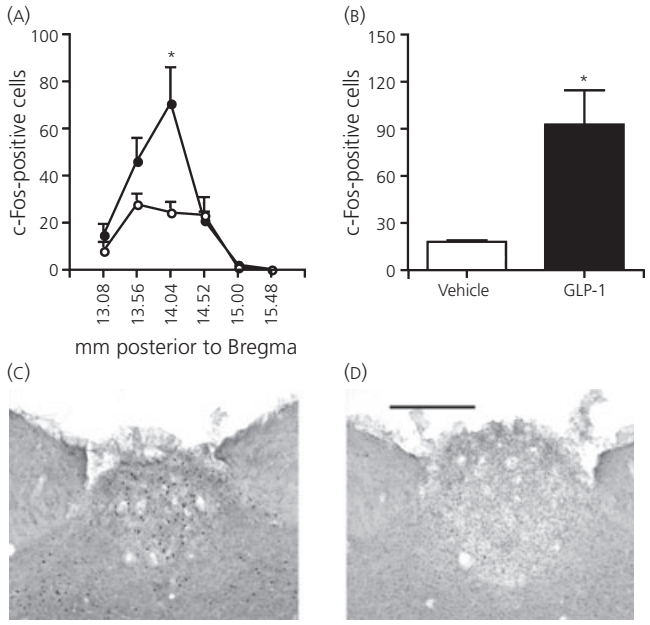

Fig. 2. Hepatic-portal vein infusions of 1 nmol/kg glucagon-like peptide-1 (GLP-1) (filled symbols) increased the number of c-Fos-positive cells in the nucleus tractus solitarii (NTS) (A) and area postrema (AP) (B) compared to vehicle (open symbols) infusions. Values are the mean ± SEM of 15 rats. *$P < 0.05$. Representative examples of c-Fos staining in the NTS and AP at 14.04 mm posterior to bregma in a GLP-1-treated (C) and vehicle-treated (D) rat are presented. Scale bar = 250 μm.

The specific neuronal activation pattern reported here has not been observed in previous studies of the effects of peripheral administration of GLP-1 or GLP-1R agonists. That is, we found more activation than some previous studies using comparably low doses of GLP-1 administered via different routes (22–24) and less activation than several studies using much larger doses (25, 26) or long-lasting GLP-1 agonists (22, 27, 28). For example, Yamamoto *et al.* (27) reported that femoral vein infusion of 0.24 nmol/kg (1 μg/kg) of the potent GLP-1 agonist exendin (4–39) (Ex-4) increased c-Fos in the NTS, AP, PVN, arcuate nucleus and other areas. It is unlikely, however, that these effects of Ex-4 are directly comparable with what might be produced by HPV infusion of the GLP-1 dose that we used because GLP-1 is rapidly degraded by dipeptidyl peptidase IV, whereas Ex-4 is resistant to this enzyme and has several orders of magnitude greater biological activity than GLP-1 (29–31). For the same reason, the effects of long-acting GLP-1-albumin conjugates on c-Fos expression (22) are also of questionable relevance. There are two reports of the effects of i.p. injections of GLP-1 doses similar to ours in rats. Rowland *et al.* (23) reported that i.p. injection of 7.6 nmol/kg GLP-1 did not significantly increase c-Fos expression in the AP, PVN, arcuate nucleus or bed nucleus of the stria terminalis, and Neary *et al.* (24) failed to detect increases in c-Fos expression in the NTS, AP, PVN or arcuate nucleus after i.p. injection of 10 nmol/kg GLP-1. A higher i.p. dose, 100 nmol/kg GLP-1, did significantly increase neuronal activation in the arcuate nucleus in rats (25) and in the NTS, arcuate nucleus and PVN in mice (26). Another study in mice (32), however, failed to detect increases in c-Fos expression in these areas or in the ventromedial hypothalamus, supraoptic nucleus or CeA after i.p. injection of 900 nmol/kg GLP-1. It appears, therefore, that the effects of GLP-1 on c-Fos expression depend critically on the dose, route of administration, test conditions and, perhaps, species. In addition, as described above, i.p. and HPV GLP-1 infusions inhibit eating by recruiting different mechanisms (9), which may lead to differential central processing and, hence, differential neuronal activation.

The pattern of brain c-Fos expression after HPV GLP-1 that we observed resembles, in some respects, the effects of physiological satiation and, in other respects, the effects of more pharmacological treatments, including treatments that probably elicit some degree of visceral malaise, but is not identical to either. The pattern of increased c-Fos expression in the NTS, AP and CeA following HPV GLP-1 resembles the neuronal activation pattern after eating. Several studies in rats indicate that eating small amounts of food increases c-Fos expression in the NTS, CeA and PVN, but not in the AP (33–36). Eating larger amounts of food (e.g. in response to overnight food deprivation), however, recruits additional c-Fos expression in these areas and can lead to increased c-Fos expression in the AP (37, 38). Thus, the results obtained in the present study, with the exception of the lack of an increase in c-Fos expression in the PVN, are consistent with the hypotheses that HPV infusion of 1 nmol/kg GLP-1 has a similar effect in the brain as ingestion of a large meal and, by extension, that such meal-stimulated effects may be mediated by GLP-1 secretion. Very large meals, of course, may induce visceral malaise as well as satiation, as discussed by Rinaman *et al.* (37) and Verbalis *et al.* (39). Finally, the lack of effect of GLP-1 on c-Fos expression in the PVN may indicate that the eating-related feedback information encoded by GLP-1 under our conditions does not include information sufficient to activate the PVN, but further work would be required to confirm this.

Tests of other satiation signals provide further insight into the potential meaning of the pattern of c-Fos expression following HPV infusion of 1 nmol/kg GLP-1. For example, in a dose–response study of i.p. injected cholecystokinin (CCK-8), an intestinal peptide satiation signal, on c-Fos expression in the NTS (39), i.p. injection of ≤ 2 μg/kg CCK-8 failed to elicit c-Fos expression in the AP, whereas i.p. injection of ≥ 8 μg/kg CCK-8 clearly did. Importantly, doses of ≥ 8 μg/kg CCK-8 usually produce aversive effects (35). A study involving i.p. injection of 5 μg/kg amylin under a number of conditions (40) may be similarly interpreted: 5 μg/kg amylin failed to elicit c-Fos expression in the AP in *ad lib* fed rats, but did elicit c-Fos expression in 24 h-fasted rats, which presumably is a more stressful situation. The association of increased AP c-Fos expression after larger versus smaller meals, larger versus smaller doses of CCK, and more versus less stressful situations are all consistent with the hypothesis that our treatment, HPV infusion of 1 nmol/kg GLP-1, may have produced some degree of visceral malaise or developing malaise. It is important to note, however, that because large, voluntary meals are one of the stimuli that produce a pattern of c-Fos expression including increased c-Fos expression in the AP, it is entirely likely that, under some conditions, GLP-1 and other eating-related feedback signals may produce both satiation and mild malaise, and that the two phenomena need not be mutually exclusive. GLP-1-expressing neurones in the caudal hind-

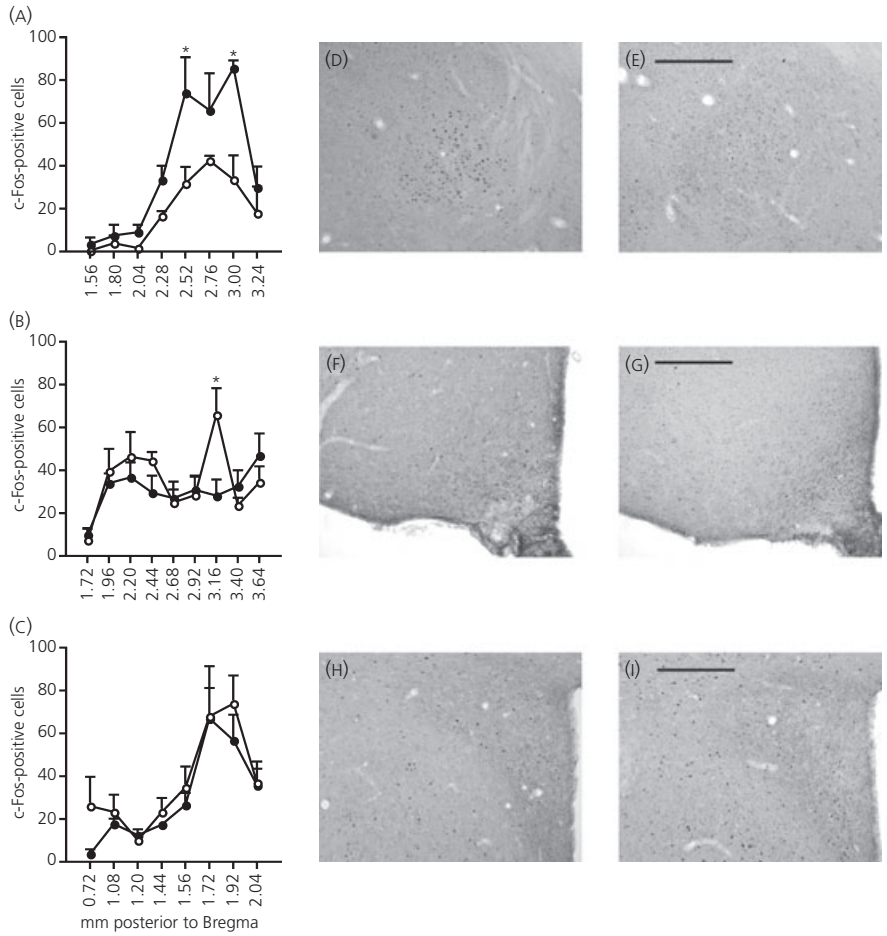

Fig. 3. Hepatic-portal vein infusions of 1 nmol/kg glucagon-like peptide-1 (GLP-1) (filled symbols) increased the number of c-Fos-positive cells in the central nucleus of the amygdala (CeA) (A), but not in the arcuate nucleus (B) and paraventricular nucleus (PVN) (C) compared to vehicle (open symbols) infusions. Values are the mean ± SEM of 15 rats. *P < 0.05. Representative examples of c-Fos staining in the CeA (D, E), arcuate nucleus (F, G), and PVN (H, I) are presented for a GLP-1-treated and vehicle-treated rat. Scale bar = 250 μm.

brain, which also abundantly express GLP-1R, are interesting to consider in this context. Rinaman (41) reported that acute visceral malaise produced by treatments such as i.p. injection of LiCl elicited c-Fos expression in caudal hindbrain GLP-1 neurones, but that very large meals (approximately 30 ml) that produced robust c-Fos expression in the AP did not. On the other hand, it appears that gastric distension-induced activation of hindbrain GLP-1 neurones has a role in normal satiation (42, 43), likely including satiation after large meals. Finally, Gaykema *et al.* (44) reported a dissociation of the effects of satiation and malaise on activation of GLP-1 neurones: ingestion of a liquid meal induced c-Fos expression in NTS GLP-1 neurones, but not in the GLP-1 neurones in the ventrolateral medulla or adjacent reticular formation, whereas i.p. injection of 100 μg/kg LPS produced the opposite pattern. A dose–response study of the effects of HPV GLP-1 on eating and c-Fos expression in hindbrain GLP-1 neurones would help to determine the role of endocrine GLP-1 in these pathways.

Although we have emphasised the eating-inhibitory actions of GLP-1 here, it is entirely possible that some or all of the c-Fos expression that we observed is related to other functions of GLP-1. In particular, GLP-1R in the hepatic-portal area have been implicated in the increases in post-prandial insulin secretion and glucose clearance that are mediated by neural reflexes with vagal or spinal-visceral afferent arms (9, 45, 46). Because both vagal and spinal-visceral afferents project to the NTS, it is possible that the increase in c-Fos expression we observed in the NTS after HPV infusions of GLP-1 was related to this function rather than to the control of eating.

Finally, it is worth noting that our eating data extend the data reported by Rüttimann *et al.* (9). Here, GLP-1 was HPV infused in rats re-fed at dark onset after 3 h of food deprivation and, under these conditions, GLP-1 reduced meal size and also increased the satiety ratio, whereas, previously (9), HPV GLP-1 infusions during truly spontaneous meals early in the dark phase reduced only meal size.

In conclusion, we have shown that GLP-1 infused via the HPV in a dose that reduced meal size increased c-Fos expression in the NTS, AP and CeA, suggesting a role for these brain areas in the eating-inhibitory action of GLP-1. Whether these effects on c-Fos expression reflect the activation of neural networks related to satiation or aversion, or both, the neurochemical phenotypes of the acti-

vated cells, and their electrophysiological characteristics require further research.

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